NTP Research Concept: Evening Primrose (*Oenothera biennis* L.) Oil

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Nomination Background and Rationale:
Evening primrose oil (EPO) was nominated for toxicological characterization by the National Institute for Environmental Health Sciences because of its widespread use in dietary supplements, lack of adequate toxicological data, and concern regarding potential adverse effects among populations that use EPO regularly. Evening primrose is a biennial weed of the Onagraceae family, native to North America and found in parts of Asia and Europe. Although the entire plant is edible; the flowers are added in salads, the leaves eaten like greens, and the roots boiled like potatoes, it is primarily a minor oilseed crop used to produce the dietary supplement. EPO consists of a variety of essential fatty acids, including γ-linolenic acid (GLA), linoleic acid (LA), oleic acid, palmitic acid, and stearic acid. It has been used to treat a variety of ailments including premenstrual syndrome (PMS), atopic eczema, psoriasis, multiple sclerosis, cancer, coronary heart disease, diabetic neuropathy, autoimmune conditions, and gastrointestinal symptoms. Of particular interest from a toxicological standpoint, EPO is also used for pre-eclampsia prevention during pregnancy, shortening and stimulating labor, and for prevention of early delivery. EPO is marketed in the United States as a dietary supplement and is classified as a "dietary supplement" under the Dietary Supplement Health and Education Act of 1994. It is produced by cold-pressing the seeds with screw presses or by extracting with hexane; the crude product is then refined. Annual global production of EPO was estimated to be between 1000 and 4000 metric tons in 2000.

Human Exposure:
EPO is commercially available in a variety of forms (e.g., capsules) via the Internet, natural food stores, drug stores, chemical companies, and other retail stores. It is used as a dietary source of essential fatty acids and in the production of soaps and ingredients in cosmetics. A 2002 survey indicated that 4.7% of the U.S. population used EPO, and its use and production appears to be growing. In the United Kingdom, EPO has been licensed for the treatment of mastalgia, PMS, and prostatitis. EPO also is approved for the treatment of atopic dermatitis and eczema in several countries but not

the United States. Recommended doses for various inflammatory diseases range from 540 mg/day to 8 g/day. Several reviews of the efficacy of EPO have been published. These reviews suggest that, with the exception of atopic dermatitis, there is not significantly strong evidence for the use of EPO for most of the indicated ailments. Side effects that have been reported are occasional headache, abdominal pain, nausea, and loose stools. Seizures have also been reported in some persons taking EPO. There were 193 adverse reactions to EPO reported from 1968-1997. The severe cases included convulsions, aggravated convulsions, face edema, and asthma.

Toxicity and Carcinogenicity Studies:
A number of studies have examined the efficacy of EPO in humans for the treatment of cancer and neurologic disorders and modulation of labor and delivery. There is little evidence of toxicity associated with these studies. A single case study describing dermal sensitization following EPO exposure has been reported. There is also an isolated report of a newborn infant whose mother self-medicated with EPO and raspberry leaf tea developing diffuse ecchymoses and petechiae following birth. The bruising cleared by 5 days of age and no further effects were reported. There are several studies indicating that EPO may improve clinical outcomes and survival in certain types of cancers in humans, but the effects are not consistent. Rodent studies also demonstrate similar inconsistencies. A high-fat diet containing 20% EPO was shown to inhibit the growth of dibenz(a)anthracene-induced mammary tumors in Sprague-Dawley (SD) rats. Similarly, a diet enriched with 5% EPO reduced the incidence of transplanted mammary tumors in Balb/c mice. In contrast, studies in C57Bl mice indicated that a diet containing 8% EPO promoted melanoma tumor growth.

A limited number of short-term, subchronic and chronic studies have been conducted in rodents following exposure to EPO. In general, no significant differences were noted in food consumption or body weight between treated and control animals. In a chronic study in male and female SD rats (100 each sex, 5-6 weeks old) administered Efamol (a commercial EPO preparation) for 53 weeks, increased potassium levels were observed in female rats consuming 2.5 mL/kg/day (Everett et al., 1988a). Testicular shrinkage or softening was noted in 24% of male rats treated with 2.5 mL/kg/day of Efamol (versus 8% in control animals). In a cancer bioassay conducted in male and female SD rats and CD-1 mice (Everett et al., 1988b) there was no significant difference in tumor incidence between control and EPO treated animals. In this study,rats and mice received Efamol (0.3, 1.0, or 2.5 mL/kg) daily via oral gavage for 104 and 78 weeks, respectively. EPO has been shown to synergize (mercuric chloride-induced autoimmunity) or antagonize (carbon tetrachloride, cyclosporine A, ethanol and imipramine) the effects of other chemicals.

Oral administration of EPO (500 mg three times per day for 1week beginning at gestational week 37 and then 500 mg once per day until labor) for the purpose of advancing cervical ripening did not shorten gestation period or decrease the overall length of labor in nulliparous women (Dove and Johnson, 1999). Labor lasted longer in women taking EPO than those who did not and the use of EPO was associated with increases in certain active phase labor abnormalities (incidence of prolonged rupture of

membranes, protracted active phase, oxytocin augmentation, arrest of descent, cesarean delivery, and vacuum extraction). No significant differences on age, Apgar score, or days of gestation were observed between treatment and control groups. Birth weight was slightly higher in infants born to EPO treated mothers as group compared to those in the control group. Male reproductive function has been evaluated in ICR mice orally administered EPO (0.5 mL) daily for 28 days. The body weight, testis weight, and the number of complete penile insertions during a three-hour period were increased in EPO treated mice. Wistar rats fed a diet supplemented with Efamol (3% of total dietary fatty acids) from three weeks of age until mating showed no differences in parturition, birth weight, postnatal growth rate, maternal weight during pregnancy, and fetal or placenta prostaglandin E2 levels as compared to control animals (Leaver et al., 1986). In some studies EPO has been shown to decrease fetal toxicity associated with zinc deficient diets, but the effects do not seem to be consistent across rat strains.

There is no evidence in the literature to suggest that EPO is genotoxic. EPO administration reduced the number of cells with micronuclei in benzo(a)pyrene-treated (75 mg/kg body weight) male Swiss mice as compared to controls, suggesting that EPO may inhibit benzo(a)pyrene-induced DNA-adduct formation.

The metabolism of essential fatty acids in humans and laboratory animals is well understood. The process is a series of desaturation and elongation reactions starting from γ-linolenic acid and LA. LA is converted to GLA by Δ6-desaturase. GLA is then elongated by elongase to dihomo-GLA (DGLA) that then may be converted to prostaglandins or leukotrienes by cyclooxygenase 1 and 2; to 15-hydroxy DGLA by 15-lipoxygenase; or to arachidonic acid by Δ5-desaturase. Arachidonic acid may be further converted to pro-inflammatory prostaglandins, or to leukotrienes by 5-lipoxygenase. Toxicokinetic studies in humans indicate that serum levels of GLA increased following oral administration of EPO. Small increases in other fatty acids, including GLA metabolites were observed. Differences in gastric absorption and plasma concentration of GLA were observed to be dependent upon the time of day that the compound was administered. Animal tissues are more active in conversion of LA and γ-linolenic acid to longer-chain PUFAs than humans. Studies in several rodent species and cats indicate that EPO increases tissue phospholipids and triglyceride levels as well as increasing the levels of GLA and its metabolites in erythrocytes.

Consumption of dietary supplements that contain polyunsaturated fatty acids (PUFAs) alters the relative balance of the different prostaglandins and leukotrienes that regulate the inflammatory response. Consistent with this, EPO and long-chain omega-6 PUFAs produced by GLA have been shown to modulate immune function (Fritsche, 2006). Feeding with GLA decreases the Th2 cytokine and immunoglobulin G1 antibody response and reduces proinflammatory interleukin (IL)-1 and tumor necrosis factor (TNF)-alpha production. This is consistent with the reported anti-inflammatory activity of EPO. EPO reduced natural killer cell activity in weanling Lewis rats fed a diet containing 20% EPO. However, in human volunteers consuming 4g encapsulated EPO per day for 12 weeks, NK cell activity was not significantly affected. There is ongoing investigation into the proinflammatory and anti-inflammatory effects of omega-6 versus omega-3

PUFAs in inflammatory conditions, particularly autoimmune diseases. Protective effects have been observed following EPO treatment for some autoimmune diseases in rodents and humans. However, EPO treatment has also been demonstrated to enhance the clinical manifestations of others. While there is considerable evidence in the literature that PUFAs modulate immune function, no systematic evaluation of immune function has been conducted.

Key Issues:
As with other dietary supplements, a key question is what product or formulation to study. Commercial preparations vary in their fatty acid content and many of these are also supplemented with other PUFAs (such as fish oil) and/or antioxidants. Although the available toxicology data is extremely limited, there is little evidence of systemic toxicity following EPO consumption in humans or experimental animals. The pattern of use and reported effects on reproductive endpoints suggest that additional studies in this area would be warranted. Because of its potential to modulate the immune system, individuals with certain types of inflammatory diseases may represent a population that is particularly susceptible to the effects of EPO.

Proposed Approach:
The overall goal of these studies will be to characterize the subchronic and reproductive toxicity using a commercially representative sample of EPO following oral exposure in rats. A current guideline functional reproductive toxicology study in rats will be conducted to adequately address the potential effects on fertility and fecundity, based on the demonstrated evidence in the literature of effects on reproductive endpoints in humans and experimental animals. A subchronic study in B6C3F1 mice and perinatally exposed Harlan SD rats is proposed to provide information on the potential targets of EPO toxicity and inform dose selection for the RACB. Bioavailability will be examined in conjunction with the subchronic studies. An immunotoxicity screening study is proposed to clarify the immune functions and cell populations modulated by EPO and inform selection of additional models that may be of value in evaluating susceptibility.

Significance and Expected Outcome:
As is the case with most herbal remedies and dietary supplements, information on the extent of exposure is not readily available. These proposed studies would address concerns with regard to the use of this dietary supplement during pregnancy, identify affected tissues and cell populations following EPO exposure and provide much-needed information on its safety for the FDA and the public.

References:
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